J. Stagg On the mechanism of anti-CD39 therapy

Letter to Reviewers

Reviewer #1:

Suggestion# 1) Authors did not inform -and thereby short-changed the Reader- about the foundational insights from the massive body of earlier work that started the field by providing the first biochemical, immunological, genetic and pharmacological evidence that extracellular adenosine -A2A Adenosine Receptor-Intracellular cAMP signaling is immunosuppressive in a context of infectious diseases and cancer. Authors know that those earlier studies provided the motivation for the generation of good scientists to investigate the CD73 and extracellular nucleotides in immunotherapies. Suggestion #1: Authors should briefly provide the Rationale to studies of CD39/CD73 as due to the solid understanding that the molecular mechanism of extracellular nucleotides-mediated immunosuppression is triggered in extracellular adenosine-rich inflamed or cancerous tissues due to binding of extracellular adenosine to Gs protein coupled A2A and A2B adenosine receptors on immune cells and subsequent transmembrane signaling.

It is these foundational papers, that eventually attracted others to this field of immunoregulation by extracellular nucleotides, led to the novel principle of INHIBITION of adenosine—A2A for immunoenhancement in cancer therapies , created an "anti-A2-adenosinergic" industry and targeting of A2AR/A2BR and CD39/CD73.

Response: We thank the Reviewer for his constructive criticisms which undoubtedly has raised the quality of our review. As recommended, our review now begins with the following statement:
“The development of adenosine-targeting agents stems from foundational insights from a large body of biochemical, immunological, genetic and pharmacological studies demonstrating the broad immunosuppressive effects of extracellular adenosine (Nature. 2001 Dec 20-27;414(6866):916-20; Huang, Blood 90:1600-1610; Wolberg G, 1975, Science). Notably, using mice genetically deficient in adenosine A2A receptor, Sitkovsky and colleagues provided the first genetic evidence of the critical importance of the adenosinergic pathway in tumor immunity (Ohta, PNAS, 2006)”.

Suggestion #2: Authors may then illuminate the Reader by explaining that the first genetic and pharmacological evidence that extracellular adenosine is A) immunosuppressive in vivo and B) is important in regulation of immune response in vivo could NOT be obtained without focus on deletion of A2AR gene as was done by Ohta et al. This is because it was impossible to have genetic "knock-out" of extracellular adenosine or genetic "knock-out" of intracellular cAMP due to myriad of their needed for cell survival cellular functions.

Response: As recommended, our review now begins with the following statement:
"The development of adenosine-targeting agents stems from foundational insights from a large body of biochemical, immunological, genetic and pharmacological studies demonstrating the broad immunosuppressive effects of extracellular adenosine (Nature. 2001 Dec 20-27;414(6866):916-20; Huang, Blood 90:1600-1610; Wolberg G, 1975, Science). Notably, using mice genetically deficient in adenosine A2A receptor, Sitkovsky and colleagues provided the first genetic evidence of the critical importance of the adenosinergic pathway in tumor immunity (Ohta, PNAS, 2006)".

Suggestion #3: Authors should explain to the Reader that the power of the immunosuppression by extracellular adenosine-A2R-cAMP events not only because it leads to the direct inhibition of TCR-activation of T cells , but also -in addition: the Adenosine -A2R-cAMP leads to immunosuppressive transcription in surrounding cells due to the phosphorylation by PKA of cAMP element binding protein (CREB) as first proposed by SitkovskyMV. T regulatory cells: hypoxia-adenosinergic suppression and re-direction of the immune response.. Trends Immunol. 2009 Mar;30(3):10

Response: We agree with the Reviewer's suggestion that the Rationale to studies of CD39/CD73 is due to the solid understanding of the molecular mechanism of extracellular nucleotides-mediated immunosuppression. The following sentence was thus added to our manuscript:

"Adenosine-mediated immunosuppression has been extensively reviewed elsewhere (PMID: 31201720). High affinity A2A and low affinity A2B adenosine receptors induce cyclic AMP response element (CRE) dependent redirection of transcription in T cells and myeloid cells (PMID: 19201652)".

Suggestion #4:
Discussing CD39 as the target for inhibition in order to improve anti-tumor immune response cannot be limited only to anti-CD39 mAb or inhibitors. Reader should be also explained the implications of connection of Hypoxia-HIF-1alpha (-Upstream-) with CD39/CD73-Ado-A2AR-cAMP (-downstream-). **Suggestion #5**: Authors also should also explain the therapeutic promise of using oxygen and oxygenation agents as treatments to inhibit the CD39 expression and CD73 expression and CD39/CD73-mediated generation of extracellular adenosine.

Response: We agree with the Reviewer, and have added the following sentence in our concluding remarks:

”Oxygen and oxygenation agents, alone or in combination with CD39 or CD73 inhibitors, to further decrease tumor adenosine levels also constitutes another potential strategy (PMID: 25739764)”.

Suggestion #7:
Discussing prospects of therapeutic targeting CD39/CD73 Authors should inform the Reader why the targeting of individual adenosine-generating enzymes is fraught with problem of redundancies. -CD38? -Alkaline phosphatase?+++? Authors should offer their opinion IF the targeting of apyrases like CD39 and adenosine generating enzymes or CD73 has clinical promise in view of redundancy of these enzymes and comment on work on adenosine-generating CD38 and NK cells of Fabio Malavasi in JI that provided evidence for the redirection of NK toward immunosuppressive phenotype as was predicted in Sitkovsky's 2009 Trends Immunology

Response: We agree that redundancies in adenosine-generating enzymes might constitute an important limiting factor to CD39- and CD73-targeting therapies. We added the following sentence to our concluding remarks:
“Finally, it remains unclear whether targeting of individual adenosine-generating enzymes may be limited with potential redundancies. For instance, the ecto-enzyme CD38 can generate AMP from NAD+ and has been shown to participate in tumor immune escape (PMID: 30012853). Similarly, tissue-specific and non-specific alkalines phosphates may provide a source of extracellular adenosine independently of CD73.”

Reviewer #2:

Comment 1) The first two paragraphs discuss the same activity of CD39 from the ATP and Adenosine point of view. I suggest that the authors combine both paragraphs and use the first "CD39 and ATP signaling" to introduce ATP function (I would also suggest to modify the title in this sense) and, in the second paragraph, detail CD39 activity by converting ATP into Adenosine and the associated immune suppression. Otherwise the first paragraph feels truncated since CD39 activity is not described until the second paragraph "CD39 and adenosine signaling".

Response: We thank the Reviewer for this suggestion. We have now fused the 2 first sections into 1 section on “CD39 and ATP signaling”.

Comment 2) In the paragraph "CD39, NLRP3 inflammosome and pyroptosis" a sentence describing the effect of CD39 abrogation would be desirable, this paper depicts it: Cell Commun Signal. 2014 Jul 16;12:40. doi: 10.1186/s12964-014-0040-3. CD39 is a negative regulator of P2X7-mediated inflammatory cell death in mast cells

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Response: We have added the following sentence and cited the above-work accordingly:
"CD39 inhibition might induce P2X7-mediated pyroptosis of myeloid cells and increased IL-1β and IL-18 production, as suggested by CD39-deficient mice (PMID: 25184735)."

Comment 3) Page 14, last paragraph, please include reference from which this information was obtained.

Response: Done

Comment 4) + signs describing cell phenotypes should be in a superindex format

Response: Done

Comment 5) Please include the ID number of the clinical trials cited.

Response: No specific clinical trial was cited. The statement in the abstract refers to the general fact that phase 1 clinical trials are evaluating adenosine-targeting agents. If the *Journal* wishes us to include citations, we will gladly do so.

Allard D, *et al. J Immunother Cancer* 2020; 8:e000186. doi: 10.1136/jitc-2019-000186